THE BINDING OF ORGANIC IONS BY PROTEINS

by

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TABLE OF CONTENTS

INTRODUCTION

For the biochemist it has been useful to consider certain metabolic processes as enzyme-substrate combinations. Although one cannot say with certainty that these combinations do embody a mechanism for certain body processes, we can gain a better concept of these processes if we picture them as an actual binding of a small particle with a macromolecular protein. There have been many studies made recently on the binding of organic ions by proteins. The aim of these studies has been to gain new insight into the nature of the complexes formed (9,16) and to relate also these binding with body processes which occur.

The binding of sulfonamides by proteins is an example of such studies. Davis (4) has shown that the distribution of sulfonamides in various body fluids is strongly dependent on the extent of their binding by proteins. It has been pointed out also in connection with chemotherapeutic properties that the bactericidal activity of these compounds parallels their absorbability by plasma. Furthermore, it has been shown by Davis that the binding properties of plasma can be attributed to its albumin fraction. Therefore, if we are to get some idea of the binding ability of plasma and its relation to disease, we must study the binding with the albumin fraction of plasma. Klotz and Walker (11) have studied the binding of several sulfonamides by bovine serum albumin for the purpose of correlating the structure of the sulfonamide and binding by the protein.

It was the purpose of this research to determine if there was a relationship between carcinogenic activity and binding of the carcinogenic dye by the protein. It is known that certain azo dyes, such as butter yellow, will cause cancer of the liver when fed to rats (13,14). It appears that whereas one substance can interact with plasma to bring about a reaction which would be beneficial to the organism, another substance might bring about a change which is detrimental. The three dyes originally selected for this study were p-aminoazobenzene, p-methylaminoazobenzene and p-dimethylaminoazobenzene (butter yellow). Of these dyes the two mentioned last are known to have considerable carcinogenic activity (14).

The binding of these dyes was determined by the dialysis equilibrium method (10,12). It was suspected, early in the problem, that troubles would be encountered since all three of the dyes are only slightly soluble in water. Therefore, to determine the effect of increasing bulk at the amino nitrogen, it appeared desirable to use the sulfonic acid derivatives of these dyes. Although binding of the sulfonic acid derivatives might not hold for the binding of the original dyes, some coherent conclusion might be reached by the study.

EXPERIMENTAL

Preparation of Dyes

p-Aminoazobenzene (6). Ten grams of aniline and 36 ml of concentrated hydrochloric acid were added to 100 ml of water. The mixture was cooled to 0°C, and a cold solution of 7.4 g of sodium nitrite was added. To this mixture 10 g of aniline in 50 ml of water with just enough hydrochloric acid to bring it into solution were added. Then 50 g of sodium acetate was added in a small amount of water. The intermediate, diazoaminobenzene, separated out as a bright yellow solid. After thirty minutes the precipitate was filtered. Yield, 13.3 g (60 percent). The intermediate was rearranged by dissolving the above yield in 30 g of aniline and adding 6 g of aniline hydrochloride. This mixture was heated at 45°C for one and one-half hours. Water was added, then dilute acetic acid until only a solid mass remained in the bottom of the flask. This was filtered and concentrated hydrochloric acid was added until most of the mass went into solution in the form of the hydrochloride. The filtrate was then treated with concentrated ammonia water and the dye precipitated. The dye was recrystallized twice from an ethyl alcohol-water mixture, then twice from a benzene-petroleum ether mixture. Finally the dye was again recrystallized from an ethyl alcohol-water mixture. Yield, 4 g (19 percent over all). Observed melting point, 122-123°C. Literature, 126°C (1).

p-Dimethylaminoazobenzene (butter yellow). This dye was obtained from Eastman Kodak Company (Technical grade). The dye

was recrystallized from an ethyl alcohol-water mixture until a constant melting point was obtained. Observed melting point, 115-115.5°C. Literature, 117(115°C) (1).

4'-Aminoazobenzene sulfonic acid-4 ("amino orange"). This compound was obtained from the Eastman Kodak Company in the form of the sodium salt (Technical grade). To determine the purity of this product the free sulfonic acid was formed by heating the sodium salt with an excess of concentrated hydrochloric acid for several hours. The dye was then filtered, washed several times and then dried. The neutral equivalent was determined potentiometrically and the following result was obtained:

	Calculated	Observed
Neutral equivalent	277	277

4'-Methylaminoazobenzene sulfonic acid-4 ("mono Methyl orange") (2). The diazotized sulfanilic acid was prepared by the method described by Fieser (5). Twenty-two g of sulfanilic acid was taken into solution by heating on a steam bath with 125 ml of water and 6.75 g of sodium carbonate. The solution was cooled and 7.4 g of finely ground sodium nitrite was added. This solution was then poured into a beaker containing 30 g of ice and 5.5 ml of concentrated sulfuric acid. The solid diazotized sulfanilic acid separated out and was filtered. The solid was then added in small amounts to a solution of 6.8 ml of concentrated hydrochloric acid in 30 ml of water. The temperature was kept below 20°C and the purple dye precipitated out in the form of the sulfonic acid. The dye was filtered and

converted to the yellow sodium salt by means of concentrated sodium hydroxide. Again the dye was filtered and placed in a Sohxlet extractor. It was refluxed overnight with benzene to remove any impurities. The benzene was removed and replaced with water. After refluxing overnight the sodium salt separated out in the bottom of the solvent flask and was filtered. Yield, 8.65 g (21.7 percent). Then excess concentrated hydrochloric acid added to convert the sodium salt back into the free sulfonic acid. The acid was filtered and washed with water to get rid of the residual hydrochloric acid. The neutral equivalent was determined potentiometrically and the following results were obtained:

	Calculated	Observed
Neutral equivalent	291	298
		284

In view of the accuracy of measurement of the base, the results obtained do not seem too out of line.

Azobenzene sulfonic acid-4. This dye was prepared by Dr. R. K. Burkhard from a procedure given in "Biochemical Preparations", I, 15. Following the sulfonation of azobenzene the product was isolated in the form of the trihydrate. The neutral equivalen was taken and found to be 316. Calculated value-316.

4'-Dimethylaminoazobenzene sulfonic acid-4 (methyl orange). The methyl orange that was used was reagent grade.

4'-Dibutylaminoazobenzene sulfonic acid-4 ("butyl orange"). This dye was prepared by Dr. R. K. Burkhard by the coupling of

dibutyl aniline and diazotized sulfanilic acid. The compound decomposed at 204°C. Literature, 198°C (15).

Preparation of Buffers

The two buffers that were used were made up to have pH values of 6.8 and 7.35, respectively. The former was prepared by weighing out 7.9 g of disodium hydrogen phosphate and 6.0 g of potassium dihydrogen phosphate and diluting to one liter. The latter was prepared by weighing out 22.8 g of disodium hydrogen phosphate and 6.8 g of potassium dihydrogen phosphate and diluting to one liter. Both of the salts that were used were anhydrous.

Determination of Dry Weight of Protein

A small sample of the protein was weighed out in a previously weighed crucible. The protein was dried in an oven at 110°C for three hours and then cooled and weighed. The loss in weight represents the water and allows calculation of the percentage of protein. Two lots of protein were used. Lot A contained 95.66 percent protein and lot B contained 93.84 percent protein.

Determination of Absorption Spectra

The determination of the spectra of the dye and its protein complex was made as follows. For a normal spectrum, the buffer was placed in one cell for a blank, and the solution of dye in buffer in the other cell. For the dye complexes, 0.050 g samples of bovine serum albumin were first placed in two test tubes. Then 25 ml of buffer were added to one tube and 25 ml of dye solution was added to the other. These solutions were shaken to get a homogenous solution. The buffer-protein solution was used as a blank for the dye-protein solution. The spectrum obtained was that of the dye-protein complex.

All spectral work was done on the Beckman Model DU quartz spectrophotometer using the 1 cm Corex cells in the visible region or the 1 cm quartz cells in the ultraviolet. The instrument was kept constant at maximum sensitivity and the slit width was varied to cause the needle to return to the null point. By this method the minimum slit width was used at all wave lenghts. Since the Beer-Lambert law holds, optical densities can be converted into molecular extinction coefficients or concentrations via the following equation

$$\text{Optical Density} = \log I_0/I = \epsilon cl$$

where ϵ is the molecular extinction coefficient, c is the concentration in moles/liter, I_0 is the intensity of the incident light, I is the intensity of the transmitted light and l is the length of the light path expressed in centimeters.

Binding Technique

The dialysis-equilibrium technique was used for quantitative determinations of the amount of dye bound. A 0.2 percent solution was placed in a cellulose bag and immersed in the dye to be bound. The tube containing the dye and the bag were shaken from 18 to 24 hours in a constant temperature bath (either at $0^{o}C$ or $25^{o}C$). The bag was removed and the concentration of the dye outside the bag was determined spectrophotometrically. The concentration of the dye was determined from a previously determined extinction coefficient or a calibration curve. The amount of dye outside the bag permits calculation of the amount of dye bound by the protein.

The following is an example of a binding run. The cellulose bags to be used were soaked in water for at least three hours, rinsed with the buffer and then allowed to stand in the buffer. Glass threads, which are used to suspend the bags in the tubes, were cut 8-10" long by means of a flame. A flame was used so there would be no loose ends in the outside solution.

The protein solution was prepared by weighing out 0.050 g of bovine serum albumin and 25 ml of buffer was added and mixed thoroughly. Since the protein is surface active, bubbles may be formed during mixing but may be destroyed by using a pipet probe coated with a thin layer of wax. The dye solutions were prepared by proper dilution of a stock solution.

Twenty ml of buffer or dye was then measured into a large pyrex test tube. A casing was then removed from the buffer and stripped between two fingers to eliminate the maximum amount of buffer. A knot was tied on one end and pulled tight. Another knot was tied below this one to make sure that no solution escapes from the bag. Then five ml of protein solution were placed in the bag by means of a volumetric pipet. The protein solution was then compressed into as small a volume as possible and the tube sealed with two knots. The excess casing at each end of the bag was cut off about 0.05" from the knot. The glass thread is tied between the knots and the bag was suspended in the test tube.

For each bag prepared with protein another one was prepared with buffer only. Both bags were placed in tubes containing the same concentration of dye. The bag with the buffer only serves as a control for the amount of dye bound by the casing. Two more bags were set up to eliminate a small error due to the bag itself. However, these two bags were immersed in buffer and not a solution of the dye.

The test tubes were then placed in the Burrell shaker in the constant temperature bath and shaken for 18-24 hours (usually overnight). The bags were removed and the concentration of the dye was determined by means of the spectrophotometer. The tube which had the buffer on the outside and the protein bag served as a blank for all those tubes which had a protein bag. Likewise the tube which had the buffer on the outside and the buffer bag served as blank for all those tubes containing buffer. The densities were taken at the absorption maximum for

the particular dye used. The densities were converted to concentrations by the extinction coefficient or a calibration curve. This is expressed in moles/liter and since the actual number of moles is desired, the concentration is multiplied by the factor of 0.025. The amount of dye bound was then found by subtracting the concentration of the dye in the protein bag tube from the concentration of the dye in the buffer bag tube. The quantity r (the moles of dye bound per mole by protein) was then found by dividing the amount of dye bound by the amount of protein present in five ml of 0.2 percent protein solution (14.4 x 10-8).

Although this method has many advantages it has also several disadvantages. It is possible that small tears are made in the bag when it is tied. Another error results because all the water cannot be stripped out of the casing. Both of these errors undoubtedly contribute to the occasion where a point is sometimes very far off the curve.

Analysis for Dyes not Obeying Beer's Law

Several of the dyes which were used in the form of the free amine did not obey Beer's law. However, it is known that dyes do obey Beer's law when they are in an acid solution. Hence the following procedure was used to analyze for p-aminoazobenzene and p-dimethylaminoazobenzene.

The extinction coefficient of the dye in known concentration was determined as follows. The dye was weighed into a 100 ml

flask, 25 ml of buffer, then 20 ml of concentrated hydrochloric acid were added. The solution was then diluted to 100 ml with 95 percent ethyl alcohol. If this was too concentrated to go on the spectrophotometer, an aliquot of the solution was taken and diluted to a known volume with a mixture of eleven parts 95 percent ethyl alcohol, four parts concentrated hydrochloric acid and five parts buffer. The reading was then taken with a blank of the above mixture. The extinction coefficient was computed from these data.

After the binding run the bag was removed and 5 ml of the dye in buffer solution was pipeted into another test tube. Then 15 ml of a mixture of four parts concentrated hydrochloric acid and eleven parts of 95 percent ethyl alcohol was added. The mixture was shaken thoroughly and the reading was taken on the spectrophotometer. The concentration of the dye was calculated from the extinction coefficient and the optical density of the dye of unknown concentration. The concentration of the dye must be miltiplied by a factor of four since 15 ml of the acid-alcohol mixture was added to 5 ml of the dye solution.

RESULTS AND DISCUSSION

Trouble was encountered with the solubility of p-aminoazobenzene and p-dimethylaminoazobenzene. The former was sufficiently soluble that a respectable binding curve could be obtained using the dialysis-equilibrium method. This curve is shown in Fig. 1.

However, the latter dye gave only a respectable yellow color when it was heated on a steam bath. When a binding experiment was run on this dye, such a large amount was bound by the bag that only a few scattered points could be obtained. Indeed the concentration of this dye was so low that there was no great difference between the optical density readings of the control and the sample. The only conclusions that can be drawn about the protein binding of p-aminoazobenzene and p-dimethylaminoazobenzene will be made from spectral changes to be discussed later.

Figure 2 shows the binding curves of the sulfonated dyes which were used in these experiments. It will be noted that azobenzene sulfonic acid is bound more strongly than any of the oranges with the exception of butyl orange. Undoubtedly the azobenzene sulfonic acid is bound through the sulfonic acid group. The binding of the oranges is also probably through the sulfonic acid group, but apparently the amino nitrogen interferes in some way to cause the binding to be less than that of the unsubstituted sulfonic acid.

There is uncertainty in the type of bond the amino residue will form with proteins. Klotz, Burkhard and Urquhart (8) have shown that the spectra of methyl orange with human serum and with bovine serum albumin are vastly different. They have shown also that by modifying the protein the sharp spectral change of human serum albumin is due to bonding involving the amino nitrogen of the dye and the phenolic hydroxyl group of the protein. Since the binding spectrum of methyl orange with

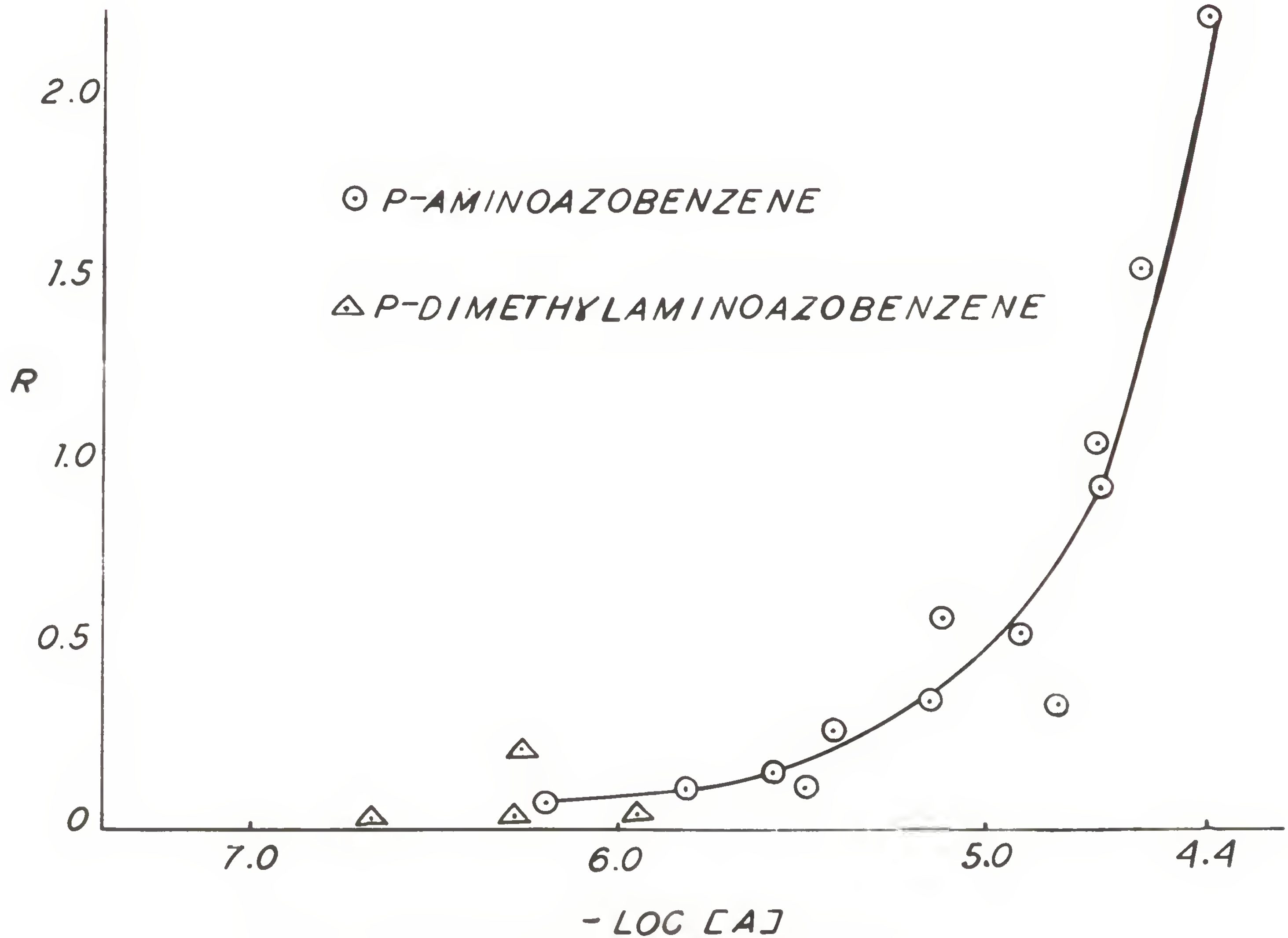

Fig. 1 Binding curve for p-aminoazobenzene

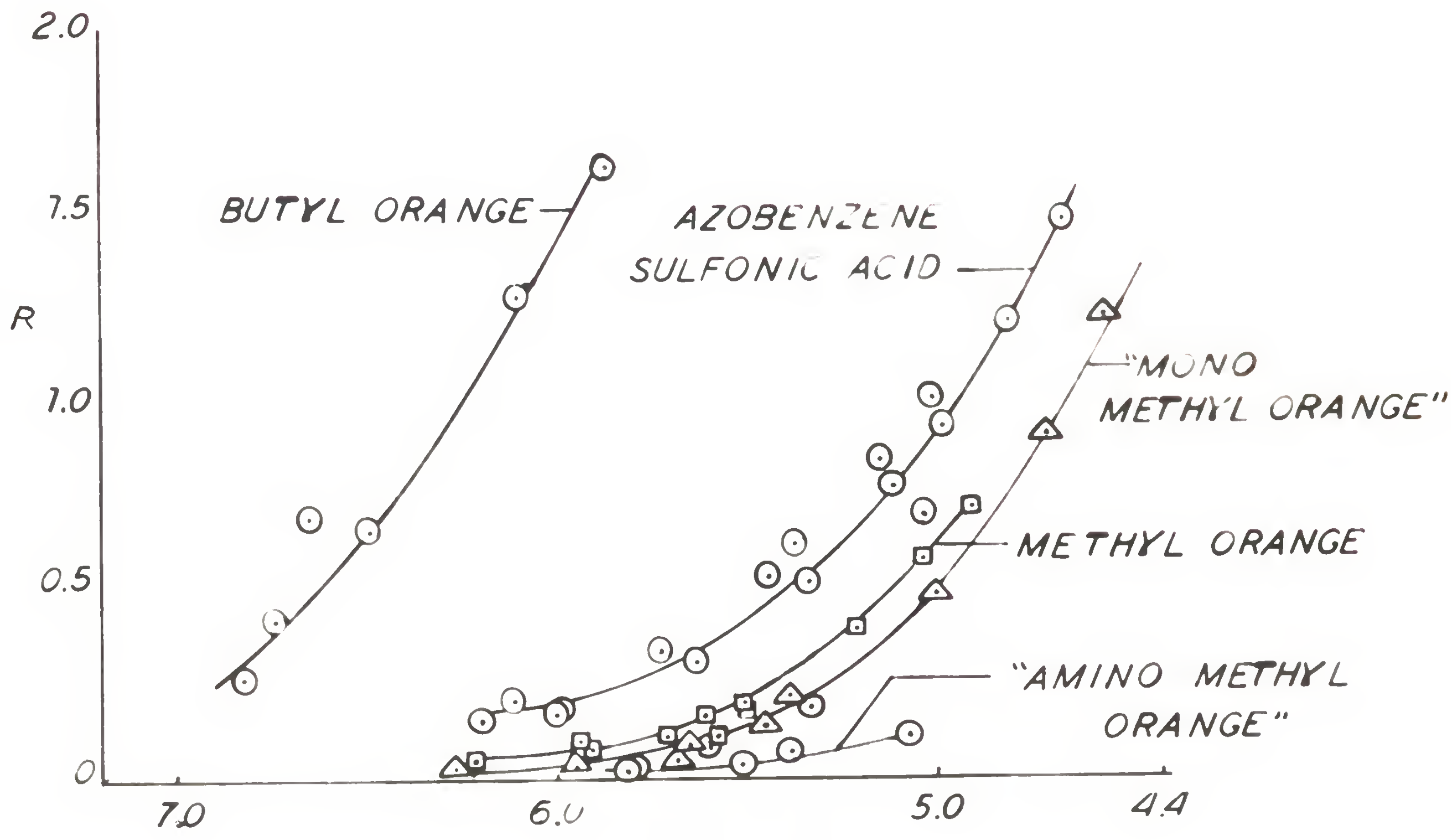

Fig. 2 Comparison of binding of sufonated dyes

bovine serum albumin does not show this type of change such binding is probably not taking place here.

If this is true then some other explanation must be given for the binding of the nitrogen substituted derivatives of 4'-aminoazobenzene sulfonic acid-4 ("amino orange"). It was thought first that the lowering of the binding of these dyes might be due to dipolar ion formation. Teresi and Luck (16) have shown that the introduction of a polar group into a molecule lowered considerably the binding of that molecule by the protein. Solubility studies were made on "amino orange", "mono methyl orange" and methyl orange to determine if the increase in the bulk at the amino group had any effect on the formation of the dipolar ion. The solubilities of these dyes were found to be respectively 4.75×10^{-4}, 5.06×10^{-4} and 2.51×10^{-4} moles/liter. Since the general trend in the solubilities does not parallel their binding no definite conclusions can be reached regarding the effect of the dipolar ion.

Another factor which might cause the binding to increase from "amino orange" to methyl orange might be the actual increase in bulk around the amino group. This increase in bulk would increase the attraction between the substituents themselves and the attractive groups of the protein. This view would tend to support the theory that the binding of the dye by the protein is through a non-polar side chain. This would be binding of the van der Waals type and not the hydrogen bonding as exhibited in the binding of methyl orange by human serum albumin.

This second explanation is supported by the fact that butyl orange is bound more strongly than azobenzene sulfonic acid. This is shown by Fig. 2. This explanation seems reasonable since Teresi and Luck (16), in their work with aromatic carboxylic acids, found that the larger the chain, the more strongly the molecule was bound by the protein. The van der Waals type of binding is convincingly supported by the data obtained by Burkhard (3) with butyl orange at 0°C and at 25°C. The curves are shown in Fig. 3. These curves show that the binding of butyl orange at 25°C is much greater than the binding at 0°C whereas the binding of azobenzene sulfonic acid at 0°C and 25°C are very nearly the same. The several types of binding which may take place between the substituted amino group and the protein, are electrostatic binding, the binding due to van der Waals forces and hydrogen bonding. Of these three one would suspect that the electrostatic binding would be the least affected by the uncoiling of the protein with increase temperature since the negative and positive charges have electric fields. Figure 3 shows the binding of azobenzene sulfonic acid does not change much with temperature whereas the binding of butyl orange is very much dependent upon temperature.

Klotz (7) has put forth another aspect of the binding of larger ions. He stated that the ability of the ion to release water molecules from the ion-protein complex is more important than increased van der Waals interactions. If the equation for complex formation can be written as $P + I = PI$, a negative entropy effect would be expected. However, just the opposite

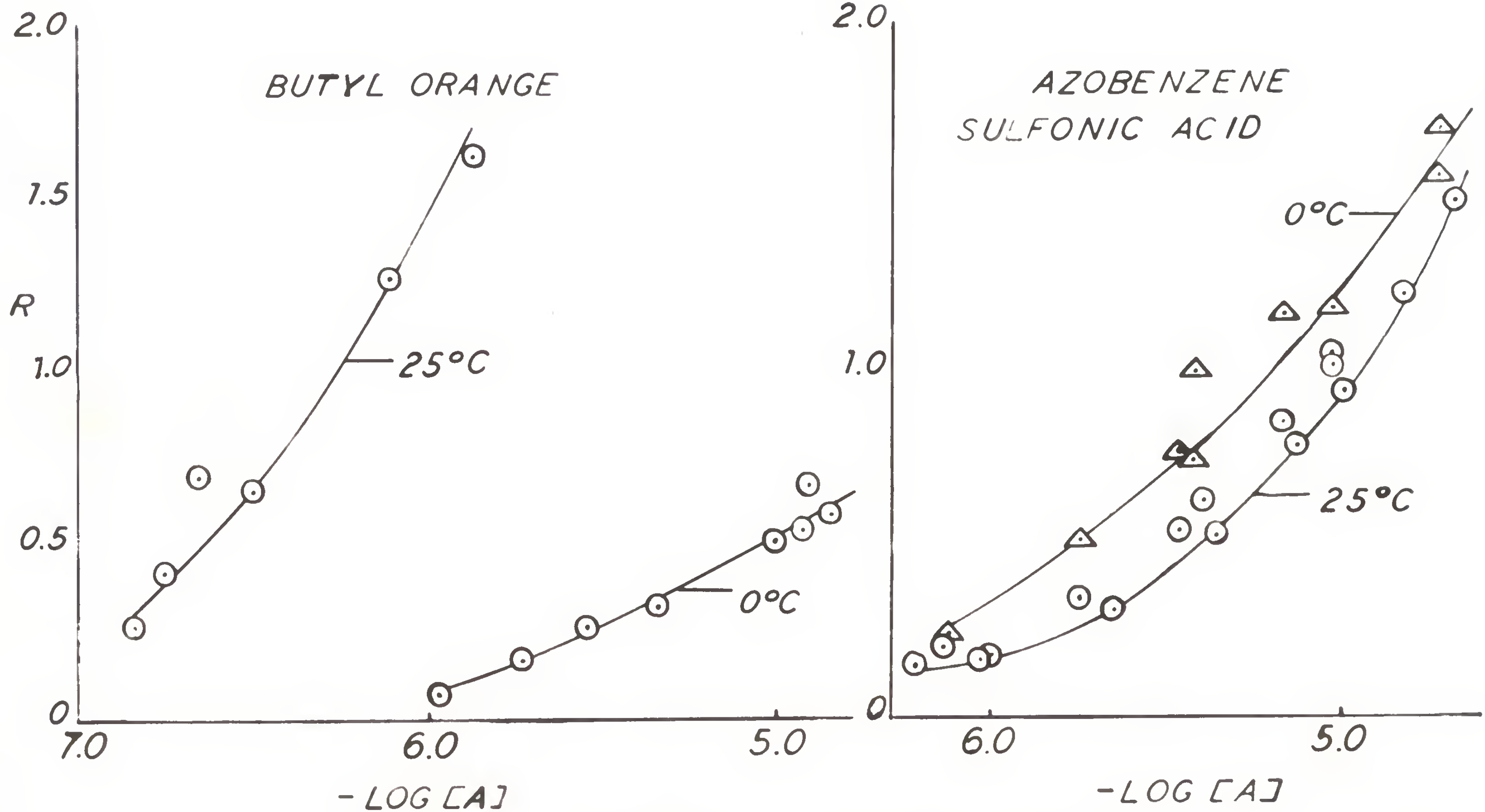

Fig. 3 Effect of temperature on binding of butyl orange and azobenzene sulfonic acid.

effect is observed in the complex formation. Klotz points out that both the ion and the protein have molecules of water attached to them. Therefore, there is a binding of the ion to the protein there is a release of water, an increase in the number of species and, hence, a positive entropy. In support of this observation Lundgren (1945) found that when an alkyl sulfonate anion is bound by egg albumin there is an increase in the volume of the solution.

The binding curve for p-aminoazobenzene and the few points obtained from p-dimethylaminoazobenzene are shown in Fig. 1. The spectral changes for the two dyes with bovine serum albumin are shown in Fig. 4. From this spectral data the change in extinction coefficient for the two dyes at equal concentrations are about the same. However, since a concentration of 3.9×10^{-6} moles/liter is about a saturated solution of butter yellow, nothing more could be gained from spectral studies. It is possible that the binding of p-dimethylaminoazobenzene is greater than that of p-aminoazobenzene and that the binding is limited only by its insolubility in the solvent used. This possibility would justify our use of the sulfonic acid derivatives in the studies of the bindings of these dyes.

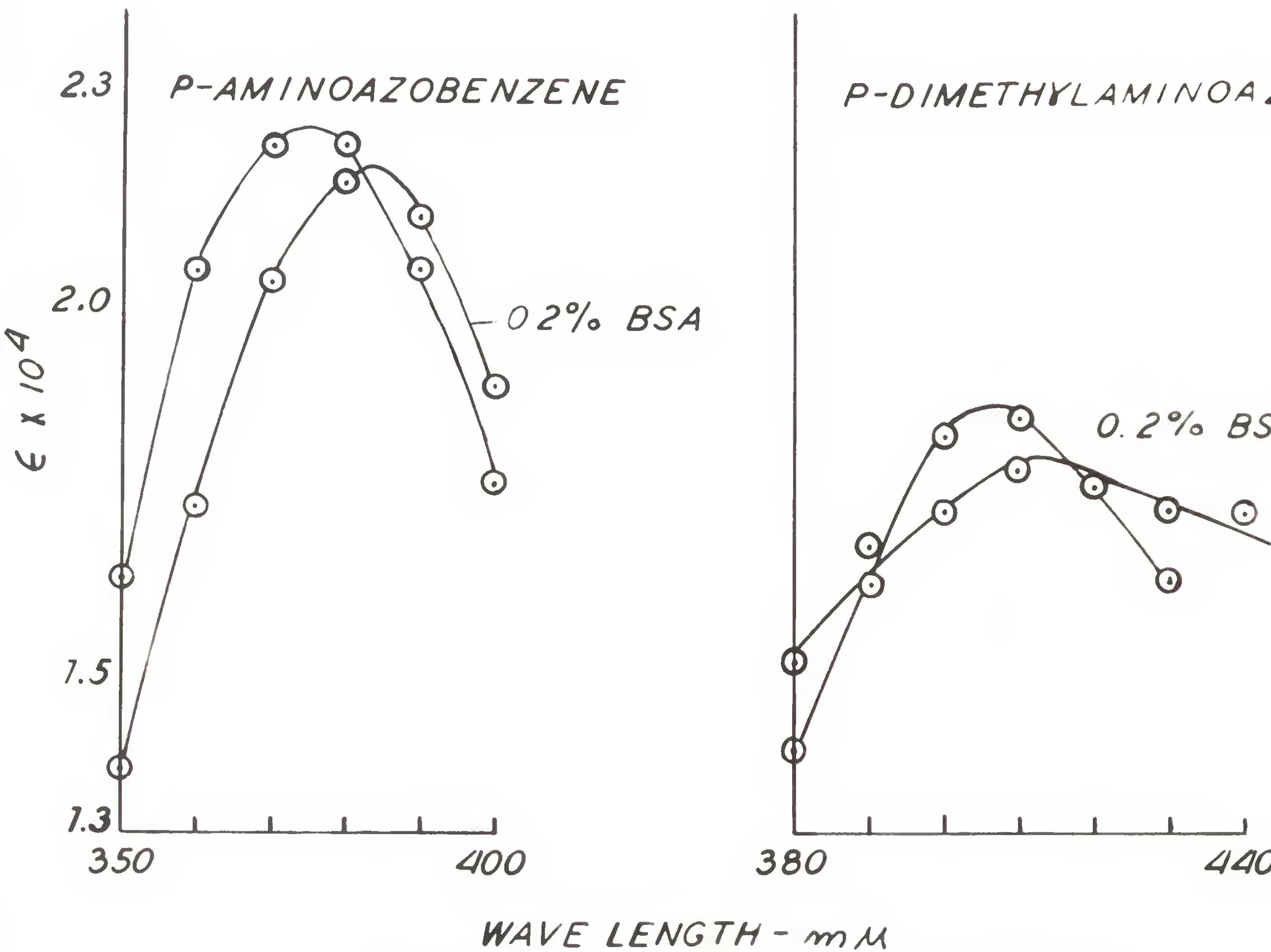

Fig. 4 Spectral changes of p-aminoazobenzene and p-dimethylaminoazobenzene.

SUMMARY

The binding of several substituted p-aminoazobenzene derivatives and their sulfonic acid derivatives by bovine serum albumin has been studied. The method used to make quantitative checks on the binding was the equilibrium dialysis method. With the sulfonic acid derivatives there was a definite increase in binding when the molecular weight on the amino nitrogen was increased. Although no definite conclusions can be made regarding the type of binding that is occurring at the amino nitrogen, temperature studies indicate the binding is not due to hydrogen bonding as exhibited by methyl orange and human serum albumin, but rather to binding of the van der Waals type where the point of attachment on the protein in a non-polar group.

p-Aminoazobenzene is bound rather strongly by the albumin, but no decent curve could be obtained for the p-dimethylamino-azobenzene. It is probably that the low binding of the latter dye is due to its insolubility in water.

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APPENDIX

Binding p-aminoazobenzene-BSA pH 7.35 25°C

Tube	Conc. M/L x 10^{-6}	Conc. M x 10^{-8}	Am't Bound M x 10^{-8}	Protein M x 10^{-8}	r	log A
1 1p	1.35 0.97	13.5 9.70	3.80	13.6	0.28	-5.411
2 2p	0.54 0.38	5.40 3.80	1.60	13.6	0.118	-5.818
3 3p	0.27 0.162	2.70 1.62	1.08	13.6	0.079	-6.188
1 1p	6.52 5.06	65.2 50.6	14.6	13.6	1.07	-4.693
2 2p	2.75 1.94	27.5 19.4	8.10	13.6	0.595	-5.112
3 3p	0.97 0.81	9.7 8.1	1.6	13.6	0.118	-5.49
1 1p	2.16 1.67	21.6 16.7	4.9	13.6	0.36	-5.168
2 2p	0.86 0.647	8.6 6.5	2.15	13.6	0.158	-5.587
3 3p	0.54 0.38	5.4 3.8	1.6	13.6	0.118	-5.818
1 1p	13.54 10.47	135.4 104.7	30.7	13.6	2.26	-4.38
2 2p	6.52 5.23	65.2 52.3	12.9	13.6	0.95	-4.68
3 3p	3.88 3.13	38.8 31.3	7.5	13.6	0.55	-4.902
1 1p	17.7 13.8	177. 138.	39.0	13.8	2.83	-4.258
2 2p	8.9 6.74	89.0 67.4	21.6	13.8	1.565	-4.57
3 3p	4.48 3.99	44.8 39.9	4.9	13.8	0.355	-4.797

Binding p-dimethylaminoazobenzene-BSA pH 7.35 25°C

Tube	Conc. M/L x 10-7	Conc. M x 10-8	Am't Bound M x 10-8	Protein M x 10-8	r	log A
1	3.6	3.6				
1p	2.83	2.83	0.67	13.6	0.049	-5.95
2	1.8	1.8				
2p	1.29	1.29	0.51	13.6	0.0375	-6.29
3	1.03	1.03				
3p	0.515	0.515	0.52	13.6	0.0382	-6.686
4	4.37	4.37				
4p	1.28	1.28	3.09	13.6	0.227	-6.29

Binding "amino Orange "-BSA pH 7.35 25°C

Tube	Conc. M/L x 10^{-6}	Conc. M x 10^{-8}	Am't Bound M x 10^{-8}	Protein M x 10^{-8}	r	log A
1 1p	9.30 8.68	23.25 21.7	1.55	13.6	0.114	-5.066
2 2p	4.41 4.13	11.25 10.32	0.93	13.6	0.068	-5.384
3 3p	1.61 1.565	4.02 3.91	0.11	13.6	0.008	-5.806
1 1p	5.6 4.56	14.0 11.4	2.6	13.6	0.191	-5.332
2 2p	2.94 2.515	7.35 6.29	1.06	13.6	0.077	-5.6
3 3p	1.185 1.138	2.96 2.84	0.12	13.6	0.009	-5.944
1 1p	3.22 3.08	8.05 7.7	0.35	13.6	0.026	-5.512
2 2p	1.71 1.66	4.27 4.15	0.12	13.6	0.009	-5.78

Binding "mono-methyl orange"-BSA pH 7.35 25°C

Tube	Conc. M/L x 10^{-7}	Conc. M x $10^{-[illegible]}$	Am't Bound M x 10^{-8}	Protein M x 10^{-8}	r	log A
1 1p	43.1 35.55	10.77 8.89	1.88	13.6	0.138	-5.45
2 2p	23.1 20.9	5.77 5.22	0.55	13.6	0.04	-5.68
3 3p	7.11 5.33	1.78 1.33	0.45	13.6	0.033	-6.273
1 1p	52.4 40.4	13.1 10.1	3.0	13.6	0.22	-5.394
2 2p	27.1 22.25	6.77 5.56	1.21	13.6	0.089	-5.653
3 3p	21.89 11.12	3.22 2.78	0.44	13.6	0.033	-5.954
1 1p	35.9 29.15	89.7 72.9	16.8	13.6	1.23	-4.536
2 2p	25.1 19.99	62.7 50.0	12.7	13.6	0.92	-4.7
3 3p	12.85 10.18	32.12 25.45	6.67	13.6	0.49	-4.99

Binding methyl orange-BSA pH 7.35 25°C

Tube	Conc. M/L x 10^{-6}	Conc. M x 10^{-8}	Am't Bound M x 10^{-8}	Protein M x 10^{-8}	r	log A
1 1p	16.6 12.6	41.5 31.5	10.0	13.8	0.725	-4.9
2 2p	12.57 9.34	31.4 23.3	8.1	13.8	0.586	-5.03
3 3p	8.38 6.18	20.9 15.4	5.5	13.8	0.399	-5.209
4 4p	4.27 3.15	10.68 7.88	2.8	13.8	0.203	-5.502
1 1p	4.15 3.15	10.38 7.88	2.5	13.8	0.18	-5.502
2 2p	3.39 2.47	8.48 6.18	2.3	13.8	0.167	-5.607
3 3p	2.55 1.95	6.37 4.87	1.5	13.8	0.11	-5.71
4 4p	1.67 1.12	4.17 2.8	1.37	13.8	0.099	-5.95
1 1p	3.23 2.63	8.07 6.58	1.49	13.8	0.108	-5.58
2 2p	1.67 1.24	4.17 3.10	1.07	13.8	0.0776	-5.906
3 3p	0.838 0.398	2.09 1.49	0.60	13.8	0.044	-6.224

Binding azobenzene sulfonic acid-BSA pH 7.35 25°C

Tube	Conc. M/L x 10^{-6}	Conc. M x 10^{-8}	Am't Bound M x 10^{-8}	Protein M x 10^{-8}	r	log A
1 1p	30.6 22.35	76.5 55.87	20.63	13.8	1.49	-4.651
2 2p	22.75 15.98	56.87 39.95	16.92	13.8	1.22	-4.796
3 3p	15.70 9.91	39.25 24.77	14.48	13.8	1.05	-5.004
4 4p	15.58 9.97	38.95 24.92	14.03	13.8	1.02	-5.002
5 5p	11.56 7.67	28.9 19.17	9.73	13.8	0.705	-5.115
6 6p	7.67 4.72	19.17 11.8	7.37	13.8	0.53	-5.326
7 7p	12.16 7.85	30.4 19.6	10.8	13.8	0.783	-5.105
8 8p	6.67 3.66	16.67 9.15	7.52	13.8	0.545	-5.437
1 1p	4.07 2.36	10.17 5.9	4.27	13.8	0.31	-5.627
2 2p	2.655 2.185	6.64 5.46	1.18	13.8	0.085	-5.66
3 3p	1.947 1.00	4.87 2.5	2.37	13.8	0.172	-6.0
4 4p	1.533 0.148	3.83 1.62	2.21	13.8	0.16	-6.188
1 1p	3.83 1.89	9.57 4.72	4.85	13.8	0.351	-5.724
2 2p	2.01 1.06	5.03 2.65	2.38	13.8	0.172	-5.975
3 3p	1.95 0.767	4.87 1.92	2.95	13.8	0.214	-6.115

Binding azobenzene sulfonic acid-BSA pH 7.35 25°C (concluded)

Tube	Conc. M/L x 10^{-6}	Conc. M x 10^{-8}	Am't Bound M x 10^{-8}	Protein M x 10^{-8}	r	log A
1 1p	15.63 11.02	39.08 27.55	11.53	13.6	0.837	-4.968
2 2p	11.98 7.26	29.95 18.15	11.80	13.6	0.855	-5.139
3 3p	7.78 4.31	19.45 10.77	8.68	13.6	0.628	-5.366

Binding "butyl orange"-BSA pH 7.35 25°C

Tube	Conc. M/L x 10^{-6}	Conc. M x 10^{-8}	Am't Bound M x 10^{-8}	Protein M x 10^{-8}	r	log A
1 1p	8.23 0.802	20.57 2.01	18.56	14.4	1.29	-6.096
2 2p	4.16 0.326	10.4 0.82	9.58	14.4	0.665	-6.487
3 3p	1.72 0.148	4.3 0.37	3.93	14.4	0.273	-6.83
1 1p	16.95 2.67	42.37 6.67	35.70	14.4	2.64	-5.574
2 2p	10.3 1.395	25.70 3.49	22.21	14.4	1.64	-5.856
3 3p	4.16 0.356	10.4 0.89	9.51	14.4	0.705	-6.649
4 4p	2.49 0.178	6.22 0.45	5.77	14.4	0.428	-6.75

Binding azobenzene sulfonic acid-BSA pH 6.8 0°C

Tube	Conc. M/L x 10^{-6}	Conc. M x 10^{-8}	Am't Bound M x 10^{-8}	Protein M x 10^{-8}	r	log A
1 1p	28.45 19.88	71.12 49.7	21.42	13.6	1.57	-4.702
2 2p	29.35 19.93	73.37 49.82	23.55	13.6	1.71	-4.7
3 3p	13.58 7.08	33.95 17.7	16.25	13.6	1.18	-5.05
4 4p	13.32 6.35	33.3 17.1	16.2	13.6	1.17	-5.165
5 5p	9.68 4.01	24.2 10.25	13.95	13.6	1.01	-5.397
6 6p	7.79 3.6	19.47 9.0	10.47	13.6	0.758	-5.444
7 7p	8.2 4.01	20.5 10.25	10.25	13.6	0.743	-5.397
8 8p	4.72 1.89	11.8 4.72	7.08	13.6	0.512	-5.724
9 9p	2.12 0.767	5.3 1.92	3.38	13.6	0.245	-6.115

Binding "butyl orange"-BSA pH 6.8 0°C

Tube	Conc. M/L x 10^{-5}	Conc. Diff. M/L x 10^{-5}	Am't Bound M x 10^{-8}	Protein M x 10^{-8}	r	log A
1 1p	0.19 0.11	0.08	2.4	32.9	0.0729	-5.959
2 2p	0.19 0.11	0.08	2.4	32.9	0.0729	-5.959
3 3p	0.38 0.19	0.19	5.7	32.9	0.173	-5.721
4 4p	0.58 0.29	0.29	8.7	32.9	0.264	-5.538
5 5p	0.73 0.47	0.26	7.8	28.5	0.274	-5.328
6 6p	1.44 0.95	0.49	14.7	28.5	0.516	-5.022
7 7p	2.01 1.45	0.56	16.8	28.5	0.590	-4.839
8 8p	1.91 1.27	0.64	19.2	28.5	0.675	-4.896
9 9p	1.70 1.21	0.49	14.7	27.4	0.536	-4.917

THE BINDING OF ORGANIC IONS BY PROTEINS

by

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AN ABSTRACT OF A THESIS

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Combinations between small organic or inorganic ions and macromolecular proteins are fundamental steps in many of the basic processes in living organisms. Not only do these basic physiological processes rely on these interactions but also the action of most drugs involves the combination of these organic ions with proteins.

The albumin fraction is known to possess a very strong binding ability as compared to the other fractions of serum protein. Therefore if one is to get some idea of the effect of the combination of an organic ion with a protein, he must check the binding of the ion with the albumin fraction of the protein. It was the point of this research to see if some relationship could be drawn between the carcinogenic activity of certain azo dyes and their binding by bovine serum albumin. The three dyes that were originally planned to be studied were p-aminoazobenzene, p-methylaminoazobenzene and p-dimethylaminoazobenzene. Of these three the latter two are known to produce cancer of the liver when fed to rats.

The method used to make quantitative binding studies on the dyes was the equilibrium dialysis method. The binding of the dye by the buffer bag and the low water solubility of the three dyes in question made it desirable to resort to the sulfonic acids of the three dyes to determine the effect of increasing bulk at the amino nitrogen.

With the sulfonated dyes there was noted definitely an increase in binding of the dye by the protein as the size of the substituent on the amino nitrogen was increased. The binding of